

Appl. No. : 09/331,631
Filed : June 21, 1999

On page 10, please amend the paragraph beginning at line 4 as follows:

RJ

It will be appreciated that one skilled in the art could take a protein with known structure, alter the sequence significantly, and yet retain the overall three-dimensional shape and antimicrobial activity of the protein. One aspect of the structure that most likely could not be altered without seriously affecting the structure (and, therefore, the activity of the protein) is the content and spacing of the cysteine residues since this would disrupt the formation of disulfide bonds which are critical to a) maintaining the overall structure of the protein and/or b) making the protein more resistant to denaturation and proteolysis (stabilizing the protein structure). In particular, it is essential that cysteine residues reside on one face of the helix in which they are contained. This can best be accomplished by maintaining a three-residue spacing between the cysteine residues within each helix, but, can also be accomplished with a two-residue interval between the cysteine residues -provided the cysteines on the other helical segment are separated by three residues (i.e., C-X-X-C-X-X-C-nX-C-X-X-X-C-X-X-C where C is cysteine, X is any amino acid other than cysteine, and n is the number of residues forming a turn between the two α -helical segments). Aromatic tyrosine (or phenylalanine) residues can also function to add stability to the protein structure if they are located on the same face of the helix as the cysteine side chains. This can be accomplished by providing appropriate spacing of two or three residues between the aromatic residue and the proximate cysteine residue (i.e., Z-X-X-C-X-X-X-C-nX-C-X-X-C-X-X-X-Z where Z is tyrosine or phenylalanine).

On page 13, please amend the paragraph beginning at line 23 as follows:

RJ

As indicated above in the description of the twelfth embodiment, the invention includes within its scope the preparation of antimicrobial proteins based on the prototype MiAMP2 series of proteins. New sequences can be designed from the MiAMP2 amino acid sequences which substantially retain the distribution of positively charged residues relative to cysteine residues as found in the MiAMP2 proteins. The new sequence can be synthesised or expressed from a gene encoding the sequence in an appropriate host cell. Suitable methods for such procedures have been described above. Expression of the new protein in a genetically engineered cell will typically result in a product having a correct three-dimensional structure, including correctly formed disulfide linkages between cysteine residues. However, even if the protein is chemically synthesised, methods are known in the art for further processing of the protein to break

E3
Conclude

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undesirable disulfide bridges and form the bridges between the desired cysteine residues to give the desired three-dimensional structure should this be necessary.

E4

On page 26, the paragraphs beginning at line 4 and ending at line 15 have been amended as follows:

SDS-PAGE gel analysis of the MiAMP2a, b, and d fragment purification is shown in the second panel of Figure 9. Lane contents are as follows: lane 1, molecular weight markers; lane 2, MiAMP2a pre-induced cellular extract; lane 3, MiAMP2a IPTG induced cellular extract; lane 4, MiAMP2a Ni-NTA non-binding fraction; lane 5, MiAMP2a elution from Ni-NTA; lane 6, MiAMP2b pre-induced cellular extract; lane 7, MiAMP2b IPTG induced cellular extract; lane 8, MiAMP2b Ni-NTA non-binding fraction; lane 9, MiAMP2b elution from Ni-NTA; lane 10, MiAMP2d pre-induced cellular extract; lane 11, MiAMP2d IPTG induced cellular extract; lane 12, MiAMP2d Ni-NTA non-binding fraction; and lane 13, MiAMP2d elution from Ni-NTA.

Using the vectors described in Example 10, MiAMP2c, and 5 homologues (i.e., MiAMP2a, MiAMP2b, MiAMP2d, TcAMP1 and TcAMP2) were all expressed, purified and tested for antimicrobial activity. The approach taken above can be applied to all of the antimicrobial fragments described in Figure 4. Purified fragments can then be tested for specific inhibition against microbial pathogens of interest.

IN THE CLAIMS:

Please cancel claims 32 and 33, without prejudice.

Please amend claims 3, 11, 13, 16-18, 30, 31, 34 and 43-45 as follows:

E5 C11

3. (Amended three times) An isolated or purified protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5.

E6 C11

11. (Amended three times) A composition comprising the protein fragment of claim 1 together with an agriculturally-acceptable carrier diluent or excipient.

E7 C11

13. (Amended four Times) A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the protein fragment of claim 1 for a period sufficient to reduce the number of said microbes.

E8 C11

16. (Amended five Times) A method of preparing an antimicrobial protein, said method comprising: